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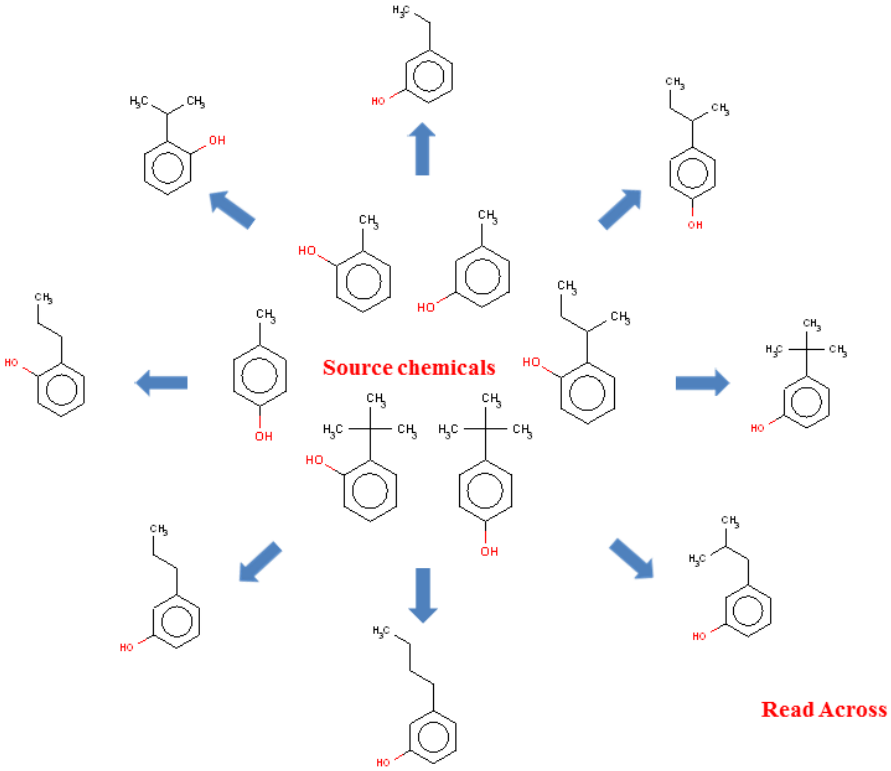
Graphical Abstract



Read-Across for Rat Oral Gavage Repeated-Dose Toxicity for Short-Chain Mono-Alkylphenols: A Case Study

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Abstract: Short-chain mono-alkylphenols provide an example of where a category-approach to read-across may be used to estimate the repeated-dose endpoint for a number of derivatives. Specifically, the NOAELs of 50 mg/kg bw/d for mono-methylphenols based on a LOAEL of very low systemic toxicity can be read across with confidence to untested mono-alkylphenols in the category. These simple alkylphenols are non-reactive and exhibit an unspecific, reversible polar narcosis mode of toxic action. Briefly, polar narcotics act via unspecific, reversible interactions with biological membranes in a manner similar to cataleptic anaesthetics. The read-across premise includes rapid and complete absorption via the gastrointestinal tract, distribution in the circulatory system, first-pass Phase 2 metabolism in the liver, and elimination of sulphates

and glucuronides in the urine. Thus, toxicokinetic parameters are considered to be similar and have the same toxicological significance. Five analogues have high quality experimental oral repeated-dose toxicity data (i.e., OECD TG 408 or OECD TG 422). These repeated-dose toxicity test results exhibit qualitative consistency in symptoms. Typical findings include decreased body weight and slightly increased liver and kidney weights which are generally without concurrent histopathological effects. The sub-chronic findings are quantitatively consistent with the No Observed Adverse Effect Level (NOAEL) of ≥ 50 mg/kg bw/d.

Chemical similarity between the analogues is readily defined, and data uncertainty associated with the similarities in toxicokinetic properties, as well as toxicodynamic properties, are low. Uncertainty associated with mechanistic relevance and completeness of the read-across is low-to-moderate, largely because there is no adverse outcome pathway or intermediate event data. Uncertainty associated with mechanistic relevance and completeness of the read-across is reduced by the concordance of in vivo, in vitro, USEPA toxicity forecaster (ToxCast) results, as well as the *in silico* data. The rat oral repeated-dose NOAEL values for the source substances can be read across to fill the data gaps of the untested analogues in this category with uncertainty deemed equivalent to results from a TG 408 assessment.

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Keywords: read-across, mono-alkylphenols, repeated-dose toxicity, No Observed Adverse Effect Level (NOAEL), Lowest Observed Adverse Effect Level (LOAEL), weight-of-evidence (WoE), uncertainty

Highlights:

- 24 short-chain (C4 or less) mono-alkylphenols were selected for read-across
- Alkylphenols exhibit an unspecific, reversible polar narcosis mode of action
- Six analogues with high quality repeated-dose toxicity data serve as source chemicals
- Uncertainty is reduced by the concordance of *in vivo*, *in vitro*, ToxCast and *in silico* data
- A NOAEL of 50 mg/kg bw/d may be read across to fill data gaps for untested analogues

1 Introduction

1.1 Read-across

Grouping of organic chemicals with the intention of conducting read-across is a method that has application in regulatory toxicology. The principal philosophy of a toxicological read-across is that chemicals that are similar in molecular structure exhibit similar chemical properties and in-so-doing, demonstrate similar toxicokinetic and toxicodynamic properties. As a consequence, experimentally-derived toxicokinetic and toxicodynamic properties from one or several substance(s), the source chemical(s), can be read across to fill the data gap for other substances, the target chemicals. This type of data gap filling is particularly useful for cosmetic ingredients, where *in vivo* testing in Europe is legislatively prohibitive [1].

Read-across arguments can be used for different purposes. The style of the read-across often differs with purpose. A wide-domain style is typically associated with screening and priority setting. Wide-domain applications have multiple target chemicals, often one, but generally three or less source substances. In contrast, narrow-domain read-across exercises include ones associated with the development of a substance-specific assessment, such as with a REACH dossier. In this case study, the wide-domain approach is used.

1.2 Short-chain alkylphenols: an overview of existing knowledge

Alkyl-substituted phenols are a structurally complex group of compounds, which differ in both the substituent size and shape and positions of substitution on the phenolic ring. They are hypothesised to act as polar narcotics by way of unspecific, reversible interactions with biological membranes in a manner similar to cataleptic anaesthetics. There are sufficient *in vivo* data available and there are also *in vitro* data from ToxCast for several of the chemicals in this

class [2]. In a preliminary investigation of alkylphenols, it was revealed that *in vivo* oral repeated-dose exposure to alkyl-substituted phenols gives rise to a variety of toxicity symptoms including toxicities involving the liver, kidney, blood and whole body effects with No Observed Adverse Effect Level (NOAEL) values ranging from >100 to <10 mg/kg bw/d [3]. Moreover, experimental results of toxicokinetics parameters are inconsistent. These toxicokinetic and toxicodynamic differences increase uncertainty associated with read-across [4]. Endpoint specific factors affecting prediction uncertainties include how molecular structure impacts metabolism and clearance, as well as repeated-dose potency.

1.3 Goal and aims

From our preliminary investigation, we conclude that alkylphenols are not likely to form a single category for repeated-dose toxicity read-across. Further, we hypothesised based on bioavailability, and distribution, and mechanistic considerations, it was highly likely that a single category could be formed for mono-alkylphenols, especially short-chain (i.e., C4 or less) derivatives. It is the intent of this case study to demonstrate that short-chain, mono-alkylphenols provide a high-quality example whereby the category approach to read-across may provide predictions for filling data gaps for the oral gavage sub-chronic repeated-dose endpoint. In this scenario, the chemical category represents analogues which are non-reactive and exhibit no specific mode of toxic action, and metabolism being consistent across the domain has minimal toxicological relevance.

The particular aims in this read-across case study were: 1) the use of online ECHA registrations information as a primary guide to and source of toxicokinetic and toxicodynamic data, 2) the incorporation of sub-chronic repeated dose toxicity data and data for alkylphenols residing outside the applicability domain of the case study, and 3) the incorporation of high-through-put

screening (HTS) data in the form of ToxCast data [5,6] and of *in silico* nuclear receptor binding predictions [7]. The specific aim of using all sub-chronic repeated-dose toxicity data (e.g., data from Organization for Economic Co-Operation and Development (OECD) test guidelines (TG) 408, TG 422 and TG 407 studies) was to increase the *in vivo* weight-of-evidence (WoE) and thereby reduce toxicokinetic and toxicodynamic uncertainties. The specific aim of the HTS data and *in silico* predictions was to reduce uncertainty associated with mechanism plausibility.

As a case study, this category assessment is designed to illustrate specific issues associated with predicting sub-chronic health effects [8]. It is not intended to be related to any regulatory discussions on this chemical group.

2 Preliminary Investigations

2.1. Toxicokinetic differences

A preliminary examination of data revealed that the alkyl substitution pattern of phenol impacts toxicokinetics. In particular, the size and number of the *ortho*-substitution impact metabolism, as does substitution in the *para*-positions. While species differences in metabolism of phenol have been shown, humans and rats showed similar metabolic pathways and quantities of metabolites in urine [9]. It was concluded that the rat is likely a good surrogate for human metabolism of phenol.

Hughes and Hall investigated the metabolism and clearance of phenol in rats [10]. The study was comparable to OECD TG 417 with acceptable restrictions. Briefly, female F344 rats (3-4/ group) received 0.03 mg/kg bw ^{14}C-labelled phenol via oral administration. Radioactivity in urine and faeces was analysed after sampling in metabolism cages; the animals were sacrificed 72 h after application and radioactivity in organs, carcass and washings determined.

Phenol showed rapid and complete absorption and was distributed throughout the body after oral exposure. Of the administered radioactivity, 70-85% of the recovered dose was excreted in urine 4 hours after administration and urinary elimination was essentially complete by 12 hours. After 72 hours, 95% of the applied dose was excreted via urine and only 1-3% was excreted via faeces. Specifically, after oral dosing 63.4 ± 2.3% was excreted as phenyl sulphate and 26.8 ± 2.7% was excreted as phenyl glucuronides. Similar findings are reported for methylphenols [11].

Takahashi and Hiraga conducted an investigation of the metabolism and clearance of 2,4,6-tritertbutylphenol in rats [12]. Clearance studies (dosed by oral gavage and in the diet) and the analysis of urinary and faecal metabolites (dosed via the diet) took place.

For clearance studies, male Sprague-Dawley rats received oral doses (260 mg/kg) of 2,4,6-tritertbutylphenol by gavage in soy bean oil following overnight starvation; rats given 2,4,6-tritertbutylphenol via the diet *ad libitum* were also used for clearance studies. At various times, rats were killed and blood, liver, spleen, kidneys, testes and samples of epididymal adipose tissue were collected for analysis. For the analysis of biliary excreted metabolites, the bile duct was cannulated with polyethylene tubing for the collection of bile. For the analysis of urinary and faecal metabolites, rats were fed a diet containing 0.2 % test material for two days, and urine and faeces were collected.

Single oral doses were well-absorbed in the rat. Peak blood levels of the test material were reached in 15 to 60 minutes. The blood elimination half-lives were 18.2 minutes for the α-phase and 11.8 hours for the slower β-phase. Maximum tissue concentrations were reached after 2 to 3 hours in the liver, 2 to 6 hours in the kidneys, 1.5 to 2.5 hours in the spleen and >24 hours in epididymal adipose tissues.

2,4,6-Tritertbutylphenol and its metabolites were not excreted in the urine; a metabolite but not the parent compound was detected in the faeces. The faecal metabolite had a molecular weight of 261 gm/mol and was considered to be a 2,4,6-tritertbutylphenoxy radical. The phenoxy radical was also detected in the bile of rats.

Several metabolic pathways and numerous metabolites of 2,6-ditertbutyl-4-methylphenol are known. The main metabolic pathway leads to the alcohol, aldehyde and acid derivatives by stepwise oxidation of the 4 -methyl group [13]. However, a cyclic metabolic pathway via quinoid metabolites (i.e., 2,6-ditertbutyl-4-hydroperoxy-4-methyl-2,5-cyclohexadienone and 2,6ditertbuty-4-hydroxy-4-methyl-2,5-cyclohexadienone) has been described in rat liver microsomes [14]. Yamamoto et al. detected reactive metabolites (i.e., 2,6-ditertbutyl-p-benzoquinone and 2,6-ditertbutylhydroquinone) possibly also a result from this pathway [15]. A further quinoid metabolite, 2,6-ditertbutyl-4-methylene-2,5-cyclohexadienone is considered to be a possible reactive metabolite [16].

Conning and Phillips studied the toxicokinetics of 2,6-ditertbutyl-4-methylphenol following oral administration [17]. For most species, hindered phenols (*ortho*-substituted) are cleared slower than unhindered phenols, due to increased enterohepatic circulation. Oxidative metabolism (i.e., phase 1 reactions) is mediated by the microsomal monooxygenase system; oxidation of the ring methyl group predominates in the rat, rabbit and monkey, while oxidation of the tertbutyl groups predominates in humans. Gallates and 2-*tert*-butylhydroquinone are the main metabolic products of non-oxidative pathways with methylation or conjugation with sulphate and glucuronic acid.

Doergea et al. studied the metabolism and disposition of isomers of 4-nonylphenol orally administrated by gavage at 0, 0, 1.25, 10 and 50 mg/kg/bw/d and by feed 50 mg/kg to Sprague-Dawley male and female rats [18]. The results showed that 4-nonylphenol was rapidly absorbed

in serum – average half-time is 0.8 hour. The aglycone content was measured in livers from rats on the 50 mg/kg diet. Tissue accumulation of 4-nonylphenol aglycone was observed despite the predominance of glucuronidation in blood. The largest difference between females and males was observed in the livers with more similar levels observed in kidney and brain. Reproductive tissues generally contained low levels of total 4-nonylphenol with the exception of prostate.

Rapid first-pass metabolism was observed and two major glucuronides were observed in rat serum and liver by LC-ES/MS analysis. Substantial amounts of p-nonylphenol-catechol glucuronides were also observed in serum and liver. The major routes of excretion of 4-nonylphenol are via the faeces.

In summary, while phenol and methylphenols are rapidly eliminated in the urine as phase 2 conjugates, 2,4,6-tritertbutylphenol and 2,6-ditertbutyl-4-methylphenol are cleared from the body more slowly. 4-Nonylphenol is also eliminated rapidly, however, the main route is via the feaces. Moreover, while phenol and methylphenols follow a single metabolic pathway, 2,4,6-tritertbutylphenol,2,6-ditertbutyl-4-methylphenol and 4-nonylphenol follows several metabolic pathways with numerous reactive metabolites being identified.

2.2 Toxicodynamic differences

A European Commission study reports data on sub-chronic oral toxicity of phenol in rats [19]. This study is considered comparable with an OECD TG 408 bioassay with restrictions (i.e., histopathology only for spleen, thymus, liver, kidneys, and male reproductive organs). Phenol was investigated for repeat-dose toxicity in male Sprague-Dawley rats exposed for 13 weeks via the drinking water at concentrations of 0, 200, 1000, 5000 mg/l (calculated to be 0, 15, 71, and 300 mg/kg bw/d). At the high dose level, decreased body weight/body weight gain, decreased

water and food consumption, and increased organ to body weight ratios were detected. It was concluded that reported effects were secondary to water avoidance due to flavour aversion. The NOAEL of phenol in drinking water was reported to be 1000 mg/l (71 mg/kg bw/d).

Sub-chronic oral toxicity studies of methylphenols (gavage dosing at 0, 50, 150, 450 mg/kg bw/d) have been reported [3]. At high doses (150 or 450 mg/kg bw/d) rats displayed lethargy, tremors, hunched posture and rough fur. There was a dose-dependent decrease in body weight or reduction in body weight increases. The NOAEL values are between 50 and 150 mg/kg bw/d.

Matsumoto et al. reports chronic oral toxicity results for 2,4,6-tritertbutylphenol from an OECD TG 452 study [20]. Briefly, 40 male and female per dose Slc:Wistar rats were exposed via the diet to 0, 30, 100, 300 and 1000 ppm for 24 months, with interim examinations at 6, 12 and 18 months. The highest dose (1000 ppm) was equivalent to approximately 1/20 of the LD50 value (1670 mg/kg in males of the same strain) obtained from a preliminary acute toxicity study. The general condition of the animals was observed and body weights were recorded throughout the study. At 6, 12, 18 and 24 months after feeding on treated diet, haematological and serum biochemical examinations were conducted for all dose groups. Also, following 6, 12, 18 and 24 months of exposure, histopathological examinations were performed for all groups.

Mortality in treated rats was comparable to that of controls (provide control mortality level or indicate no mortality). No remarkable general findings in food consumption were observed in the control and treated groups throughout the experimental period. Substantial reduction of body weight gain was found in the female 1000 ppm group from 12 months onward. No significant changes in food consumption were observed in the control and treated groups throughout the experimental period. The haematological, biochemical and histopathological examinations revealed slight microcytic anaemia, changes in some biochemical parameters relating to liver

function (e.g. phospholipids, total cholesterol, glutamate oxaloacetate transaminase and γ-glutamyl transpeptidase) and focal necrosis of liver cells following test material administration. The changes observed in females were more severe than those in males. No neoplastic response following test material administration was noted. In summary, the study concluded that 2,4,6-tritertbutylphenol causes liver injury characterised by focal necrosis with microcytic anaemia and elevations of serum phospholipids and cholesterol levels, presumably occurring as secondary effects following the liver injury. Under the conditions of this study, the LOAEL was determined to be 100 ppm (167 mg/kg bw/d); the NOAEL was determined to be 30 ppm (50.1 mg/kg bw/d).

From our preliminary investigation of available studies, we conclude that alkylphenols are not likely to form a single category for repeated-dose toxicity read-across. However, we do hypothesize it is likely that a single category could be formed for mono-alkylphenols, especially short-chain (i.e., C4 or less) derivatives. The multi-substituted alkylphenols and alkylphenols with longer (C5 or more) alkyl groups do not belong to this category because of differences in toxicokinetics.

3. Method and Materials

This evaluation of selected alkylphenols is generally consistent with the read-across workflow of Schultz et al (2015) [8]. This evaluation is also consistent with the guidance proposed by the OECD [21]. *In vivo* toxicokinetic and toxicodynamic data used in the assessment were taken from the literature and the European Chemicals Agency (ECHA) REACH Registered Substances database [22]. Mechanistic relevance, as well as toxicological similarity of the category members, was further established using relevant non-animal data.

3.1 Target and source substances

234 The short-chain mono-alkylphenols evaluated in this study are listed in Table 1. They include 19
235 potential target substances and six source chemicals (noted in bold). This list is not meant to be
236 all inclusive. Rather, it represents existing industrial organic materials that are likely to be found
237 in a governmental or industrial inventory (e.g., OECD High Production Volume Chemicals).
238 Short-chain was defined as having alkyl-substituents of C4 or less.

239 **Table 1.** Short-chain, mono-substituted, alkyl phenols evaluated in the case study.

ID	Name	CAS number
1	**2-methylphenol**	**95-48-7**
2	**3-methylphenol**	**108-39-4**
3	**4-methylphenol**	**106-44-5**
4	2-ethylphenol	90-00-6
5	3-ethylphenol	620-17-7
6	4-ethylphenol	123-07-9
7	2-propylphenol	644-35-9
8	3-propylphenol	621-27-2
9	4-propylphenol	645-56-7
10	2-isopropylphenol	88-69-7
11	3-isopropylphenol	618-45-1
12	4-isopropylphenol	99-89-8
13	2-butylphenol	3180-09-4
14	3-butylphenol	28805-86-9
15	4-butylphenol	1638-22-8
16	2-isobutylphenol	4167-75-3
17	3-isobutylphenol	30749-25-8
18	4-isobutylphenol	4167-74-2
19	**2-secbutylphenol**	**89-72-5**
20	3-secbutylphenol	3522-86-9
21	4-secbutyphenol	99-71-8
22	**2-tertbutylphenol**	**88-18-6**
23	3-tertbutylphenol	585-34-2
24	**4-tertbutylphenol**	**98-54-4**

240

241 3.2 Endpoint

The NOAEL for sub-chronic rat oral repeated-dose is the endpoint for which this category-approach to read-across is applied. The 90-day oral gavage repeated-dose data for 2-methylphenol, 3-methylphenol, and 4-methylphenol are well suited for reading across. These three analogues have been examined for toxicokinetics and the experimental NOAELs are based on experimental results from multi-dose gavage exposure scenario and following test guidelines similar to OECD TG 408 where the LOAEL symptoms are reported. The data are highly similar both qualitatively and quantitatively. Additionally, three other analogues with longer side chains (2-secbutylphenol, 2-tertbutylphenol and 4-tertbutylphenol) have the experimental NOAEL values obtained via studies following test guidance similar to OECD TG 407 and TG 422. Only one of them - 4-tertbutylphenol - was examined for toxicokinetics.

3.3 Hypothesis of the category

The initial hypothesis for this read-across case study is:

- Short-chain (i.e., C4 or less), mono-substituted alkyl phenols are chemically similar with structure and property differences that are not relevant to repeated-dose potency.
- Short-chain, mono- substituted alkyl phenols are readily absorbed from oral administration, readily distributed via the blood, similarly metabolised in the liver and readily excreted via the urine.
- Short-chain, mono- substituted alkyl phenols elicit similar qualitative and quantitative repeated-dose toxicity. *In vivo*, they exhibit no systemic toxicity. *In vitro* and *in silico*, they exhibit no chemical reactivity; nor do they exhibit any receptor-mediated interactions which are endpoint-relevant.

4 Results

4.1 Applicability domain

After elimination of multi-substituted alkylphenols and alkylphenols with large alkyl group (e.g., 4-nonylphenol) due to toxicokinetic considerations, the applicability domain was limited to mono-alkyl substituted phenols with carbon chain lengths from C1 to C4. Specifically, these derivatives included ones substituted in the 2-, 3-, or 4-position (Table 1). While data for phenol, mixtures of mono-ethylphenols, di-methylphenols and 2-isopropyl-5-methylphenol are reported and used in support of the read-across, mixtures are not included in category at this time.

4.2 Purity/impurities

A purity/impurity profile for the analogue listed in Table 1 is not reported. No effort was made to take into account impurities based on production. Since the category is structurally limited, the impurities are expected to be similar if not the same across the members and are not expected to significantly impact the toxicity profile of any analogue. However, it is acknowledged for regulatory decisions such information may be required.

4.3 Read across justification

In order to conduct a read-across, there is the requirement of high quality *in vivo* data for the endpoint under consideration, which in this case is sub-chronic oral gavage repeated dose-toxicity for rat in the form of a NOAEL value.

Table 2. Summary of repeated-dose and toxicokinetic information for selected alkylphenols.

Chemical Name	Route of administration	TG408 LOAEL (mg/kg bw/d)	TG408 NOAEL (mg/kg bw/d)	TG422 LOAEL (mg/kg bw/d)	TG422 NOAEL (mg/kg bw/d)	TG407 LOAEL (mg/kg bw/d)	TG407 NOAEL (mg/kg bw/d)	Toxicokinetic Study
2-methylphenol	Gavage	175	50					Bray et al. [23]
2-methylphenol	Diet	325	≈25					Bray et al. [23]
3-methylphenol	Gavage	150♂	50♂	1000♂	300♂			Bray et al. [23]
3-methylphenol	Gavage	450♀	150♀	300♀	100♀			Bray et al. [23]
4-methylphenol	Gavage	175	50					no
2-secbutylphenol	Gavage			60	12			no
4-tertbutylphenol	Gavage			200♂	60♂			Koster et al. [24]
2-tertbutylphenol	Gavage					500	100	no
mixture of 3- & 4-methylphenol	Diet (TG 416)	≈70	NA					Morinaga et al. [11]
mixture of 2-, 3- & 4-ethylphenol	Gavage			245	100			no
mixture of dimethylphenols	Gavage			245	100			no
Phenol	Drinking water (TG451)					300	71	Hughes and Hall [10]
2-isopropyl-5-methylphenol	Gavage			40	8			Austgulen et al. [25]

Information sourced from [22]

The sub-chronic oral repeated-dose data were collected for six alkylphenols with different length of side chain (methyl, sec-butyl and tert-butyl). Having diverse (in terms of side chain size) source chemicals adds strength to read-across justification as well as allowing for an interpolation rather than an extrapolation of data. Although there are differences in the protocols of the sub-chronic studies, the results exhibit qualitative and quantitative consistency. . Additionally, to eliminate the uncertainty that reduced food or drinking water consumption was due to flavour aversion caused by the phenols, only data from gavage studies were read across.

4.4 Similarities in chemistry

Chemical structure and property values are reported in Tables 1 – 3 of the supplementary material.

4.4.1 Structural similarity

All the alkyl phenols included in Table 1 belong to a common chemical class, phenols, and the subclasses alkyl phenols and mono-substituted phenols, and they possess a benzene backbone as common molecular scaffolding. The main structural variables in the groups are the shape and size of the alkyl-substituents and the positions of the substituents in relationship to the hydroxyl group (see supplementary material Table 3).

4.4.2 Chemical property similarity

The experimental physico-chemical properties for alkyl phenols included in Table 1 are presented in supplementary material (Table 2). Properties, with the exception of density, tread in values related to C-atom number within a scaffold. Specifically, all category members exhibit molecular weights from ≈100 to ≈150g/mol. While hydrophobicity (log Kow) increases with number of C-atoms from ≈2.00 to ≈3.30, density is constant at ≈1.0 g/cm^3. While vapour pressure and water

solubility decrease with molecular size, melting point and boiling point increase with molecular size.

In summary, the differences in chemistry observed between the analogues within the category are hypothesized to minimal and not considered to be toxicologically significant and not preclude read across for the relevant to the repeated-dose endpoint.

4.5 Similarities in toxicokinetics

Key toxicokinetic studies are listed in Table 2 and are reported in details in Sections 4.5.1 and 4.5.2. Based on the results of five toxicokinetic studies, the absorption, distribution, metabolism and elimination (ADME) properties for the short-chain, mono-substituted, alkylphenols in Table 1 are similar. All the phenols in the proposed category are predicted to be readily absorbed from the gastrointestinal tract and distributed in the blood. First pass metabolism is most likely to be phase II conjugation to glucuronides and sulphates. Elimination is predicted to be rapid (half-life of hours) and mainly via the urine. The relative amounts of specific metabolites are likely to differ between analogues (greater conjugation with higher C-number and degree of branching) and also vary with dose (greater amount of conjugation with dose assuming higher doses do not saturate).

4.5.1 Similarities in toxicokinetics of mono-alkylphenols

Morinaga et al. reported on the toxicokinetics of an oral-administered cresol soap solution containing 3-methyl- and 4-methylphenol [11]. In Wister rats, the phenols were readily absorbed, distributed throughout the body, eliminated, for the most part, within several hours and excreted mainly as glucuronide and sulphate metabolites.

Bray et al. reported the results of a single oral application of 2-methylphenol by gavage to rabbits [23]. 2-Methylphenol dissolved in bicarbonate was administered and urinary excretion was collected over 24 hours. Results showed that 80% of the dose was excreted in the urine, 55-91 % as glucuronides, 13-19 % as sulphates and 0-2 % as free 2-methylphenol.

Bray et al. reported the results of a single oral application of 3-methylphenol by gavage to rabbits [23]. Three-methylphenol dissolved in bicarbonate was administered and urinary excretion was collected over 24 hours. Results showed that the 3-methyl derivative was excreted mainly in the urine: 53-70 % (of the administered dose) as glucuronides, 4-15 % as sulphates and 0-4 % as free 3-methylphenol.

Koster et al. examined the toxicokinetics of 4-tertbutylphenol in rats. Absorption following oral administration in rats is rapid and complete [24]. Elimination of an oral dose (147 µg/kg bw/d for 3 days) was via urine and faeces, 72.9% and 26.7%, respectively. The retention of 4-tertbutylphenol in rats has been shown to be only 0.1% 7 days after an oral dosing. While urinary metabolites were sulphate and glucuronide conjugates, whether the material detected in faecal samples occurred as unabsorbed 4-tertbutylphenol or as its metabolites eliminated via bile was not determined. Following oral absorption, a small amount of 4-tertbutylphenol is distributed to the liver in rats but not to adipose tissue or lungs.

In summary, following oral exposure short-chain, mono-alkylphenols are readily bioavailable. Since the pKa of phenol and alkylphenols is between 9 and 10.6, at physiological pH, these phenols are essentially 100% non-ionized. Uncharged phenols, as compared to ionized phenols, are assumed to more readily partition across cell membranes and be absorbed in gastrointestinal tract. Metabolism is typically via phase II conjugation to glucuronides and sulphates. Elimination

is rapid (half-life of hours) and takes place mainly via the urine. The relative amounts of specific metabolites differ between analogues and also vary with the dose.

4.5.2 Supporting toxicokinetic data

The above findings are supported by the study of Austgulen et al. [25], who reported toxicokinetic data for 6 male Wistar rats exposed by gavage to a single application (1 mmol; 150.22 mg/kg bw) of 2-isopropyl-5-methylphenol dissolved in propylene glycol. Briefly, urine samples were collected and stored at -10°C in 24 hour intervals and analysed by chromatography after conjugate hydrolysis. The urinary excretion of metabolites was rapid, with only very small amounts excreted after 24 hours. The administered 2-isopropyl-5-methylphenol was excreted unchanged (or as glucuronide and sulphate conjugates). Additionally the following non-reactive metabolites were identified: 2,5-dihydroxy-p-cymene, 2-(2-hydroxy-4-methylphenyl)propan-1-ol, 5-hydroxymethyl)-2-(1-methylethyl)phenol, 2-(4-hydroxymethyl-2-hydroxyphenyl)propan-1-ol, 2-(2-Hydroxy-4-methylphenyl) propionic acid, 3-Hydroxy-4-(1-methylethyl) benzoic acid.

4.6 Similarities in toxicodynamics

4.6.1 Similarities in repeated-dose toxicity: OECD TG 408

Experimental 90-day oral gavage repeated-dose toxicity data for 2-methylphenol, 3-methylphenol and 4-methylphenol was generated by Dietz et al. [26] based on the protocol of Sontag, Page and Saffotti (NCI, DHEW Publication No. (NIH)78-ß01 Guidelines for Carcinogen Bioassay), which is similar to OECD TG 408.

Briefly, in this study 2-methylphenol diluted in corn oil was administered daily to Sprague-Dawley rats (30/sex/dose) by gavage at dose levels of 0, 50, 175, 600 mg/kg bw/d for up to 13 weeks. At

600 mg/kg bw/d significant mortality (i.e., 19/30 ♀ and 9/30 ♂) was observed. While body weights of females were unaffected, the body weight gain in males at 175 and 600 mg/kg bw/d was reduced. There was a slight decrease in food intake in the 600 mg/kg bw/d group in both sexes. Treatment-related depression of the central nervous system (i.e., lethargy, dyspnoe, tremor and/or convulsions) was observed, with recovery within 1 h after dosing. No effects on clinical chemistry, haematology, organ weights or treatment-related gross and histopathology were reported. Regardless of sex, the 90-day LOAEL was 175 mg/kg bw/d, while the NOAEL was 50 mg/kg bw/d.

3-Methylphenol diluted in corn oil was administered daily to Sprague-Dawley rats (30/sex/dose) by gavage at dose levels of 0, 50, 150, 450 mg/kg bw/d for 13 weeks. At 450 mg/kg bw male and female rats displayed lethargy, tremors, hunched posture and rough fur post-dosing. Dose-dependent body weight decrease in males at 150 mg/ kg bw/d resulted in a male NOAEL of 50 mg/kg bw/d; based on reduced body weight gain in females at 450 mg/ kg bw/d, the 90-day female NOAEL was 150 mg/kg bw/d [26].

4-Methylphenol diluted in corn oil was administered daily to Sprague-Dawley rats (30/sex/dose) by gavage at dose levels of 0, 50, 175, 600 mg/kg bw/d for 13 weeks. Based on increased mortality, clinical signs (i.e., lethargy, excessive salivation, tremor and occasional convulsions and comas), as well as hepatotoxicity and nephrotoxicity in both sexes, the LOAEL was reported as 175 mg/kg bw/d. The 90-day NOAEL was reported as 50 mg/kg bw/d for both sexes [26].

A reliable without restrictions study reports the 90-day feeding oral toxicity of 2-methylphenol [27]. Following OECD TG 408, Fischer 344 rats (20/sex/dose) were fed diets containing 0, 1880, 3750, 7500, 15000, 30000 ppm (≈ 160, 325, 650, 1300 and 2600 mg/kg bw/d) 2-methylphenol for 13 weeks. While no mortality was observed, decreased body weight gain was recorded at the

highest dose. No clinical signs of toxicity were reported. Increased relative kidney and liver weights were observed at the three highest doses; however, haematology and clinical chemistry findings were “unremarkable”. Similarly, histopathology findings were minimal and considered likely secondary to the decreased weight gains.

In summary, at higher doses (<450 mg/kgbw/d), 90-day repeated oral gavage exposure to methylphenols result in displays of lethargy, tremors, and hunched posture. Dose-dependent body weight decrease is observed at intermediate doses. Histopathology findings, when noted, were minimal and considered likely secondary to the decreased weight gains. Increased relative kidney and liver weights, when noted, are likely compensatory to metabolism. Typically, experimental NOAEL values of 50 mg/kg bw/d are reported.

4.6.2 Similarities in repeated-dose toxicity: OECD TG 422, and TG 407

A reliable with restrictions study reports findings for 2-secbutylphenol from a study design similar to OECD TG 422. Crj:CD(SD) rats (13/sex/dose group) were dosed by oral gavage to 0, 12, 60 and 300 mg/kg bw/d (corn oil carrier) [28]. Males were exposed for 42 days, females, from 14 days before mating up to day three of lactation. No animals died in any groups. In the 300 mg/kg bw/d group the following symptoms were observed: 1) salivation after dosing, decrease in activity and incomplete eyelid opening in males and females, 2) an ataxic gait was in females, 3) an increase in relative liver weight with males and females, and hypertrophy of the centrilobular hepatocytes in males, 4) concentration of total cholesterol was increased in males, and 5) no adverse effects were detected on food consumption and body weight change in males and females. In the 60 mg/kg group, decrease in locomotor activity was observed in a few males early in the

administration period. The “NOELs” for repeat dose toxicity of 2-secbutylphenol are considered to be 12 mg/kg/d in males and 60 mg/kg/d in females.

The US EPA [3] reported repeated-dose toxicity data for 4-tertbutylphenol. In a 14-day range-finding study (for the definitive study below), Sprague-Dawley rats (5/sex/dose) were administered 4-tertbutylphenol daily via gavage in 0.5% aqueous methyl cellulose at 0, 250, 500 and 1000 mg/kg bw/d. At 1000 mg/kg bw/d, mortality (3 of 5 females and 1 of 5 males) and decreased body weight were observed. Two females at this dose had difficulty breathing. No signs of toxicity were noted when the animals were necropsied. A dose of 250 mg/kg bw/d was considered an appropriate dose level for this study [3].

In a combined repeated-dose/reproductive/developmental toxicity screening test following OECD TG 422, male and female Sprague-Dawley rats (13 sex/dose) were administered 4-tertbutylphenol via gavage in 0.5% aqueous methyl cellulose at 0, 20, 60 and 200 mg/kg bw/d [29]. Males were exposed for 44 days; females were exposed from 14 days before mating to day 4 of lactation. At the highest dose tested, some females showed stridor associated with dyspnea, likely caused by irritation of the respiratory tract as a result of the gavage dosing. In the same test group, males exhibited decreased plasma albumin. In parental animals, no compound specific morphological changes were observed. Examination of body weights and gross morphology of the offspring revealed no effects of the compound administration and no other treatment-related changes were observed. A NOAEL of 60 mg/kg bw/d was reported for 4-tertbutylphenol.

A study following OECD TG 422 reported the sub-chronic oral toxicity of a mixture of 2-, 3-, and 4-ethylphenol (29%, 32% and 39%, respectively) [30]. Crl:CD (SD)IGS BR VAF/Plus rats (10/ sex/dose) were exposed by gavage in corn oil to 0, 30, 100 and 245 mg/kg bw/d. Males were exposed for 28 days and females were exposed 54 days. Observations of viability, clinical signs of

toxicity, food consumption, body weight gain, functional observational battery and motor activity, haematology, clinical chemistry, as well as gross and microscopic post-mortem examination were undertaken. All rats survived the treatment. In males, urine-stained fur was observed at the 245 mg/kg/day level. Body weight gain and food consumption were unaffected by treatment. Symptoms associated with neurotoxicity were not observed during the study, and there were no treatment related effects observed at gross necropsy or with histopathology. Due to urine-stained fur, increased kidney, liver and ovarian relative weights at 245 mg/kg bw/d, the NOAEL was reported as 100 mg/kg bw/d.

Another study following OECD TG 422 reported the sub-chronic oral toxicity of a mixture of dimethyl-substituted phenols [31]. The tested material included: 2,5-xylenol (95-87-4): 16.4 mole %, 3,4-xylenol (95-65-8): 16.9 mole %, 2,4-xylenol (105-67-9): 22.7 mole %, 3,5-xylenol (108-68-9): 11.1 mole %, 2,3-xylenol (526-75-0): 18.2 mole %, and 2,6-xylenol (576-26-1): 14.7 mole %. Crl:CD (SD)IGS BR VAF/Plus rats (10/ sex/dose) were exposed by gavage in corn oil to 0, 30, 100 and 245 mg/kg bw/d. Males were exposed for 28 days and females were exposed for 54 days. Viability, clinical signs of toxicity, food consumption, body weight gain, functional observational battery and motor activity, haematology, clinical chemistry, as well as gross and microscopic post-mortem examination were assessed. All rats survived the treatment. In males, urine-stained fur was observed at the 245 mg/kg/day level. Body weight gain and food consumption were unaffected by treatment. Symptoms associated with neurotoxicity were not observed during the study, and there were no treatment related effects observed at gross necropsy or with histopathology. Due to urine-stained fur, increased kidney, liver and ovarian relative weight at 245 mg/kg bw/d, the NOAEL was reported as 100 mg/kg bw/d.

In a reliable with restrictions study, 2-tertbutylphenol was assessed following a procedure similar to OECD TG 407 [32]. In this 28-day repeated-dose study, Crj:CD(SD)IGS rats (6/sex/dose) were administered 2-tertbutylphenol in olive oil by gavage at doses of 0, 4, 20, 100 and 500 mg/kg bw/d. No treatment-related changes in body weight, food consumption, haematology, blood and urine chemistry, urinalysis were noted. Necropsy and histopathological examination were unremarkable. Clinical signs of ataxic gait were observed in both males and females in the 500 mg/kg group. Transient salivation within 30 minutes of dosing was observed as the only clinical sign in males and females in the 100 mg/kg group. For both sexes, the NOAEL values for 2-tertbutylphenol were reported as 100 mg/kg bw/d.

Consistent with the above studies, a reliable with restrictions study reported the results of an OECD TG 451 assay using F344/N male rats that were fed a mixture containing 60 % 3-methylphenol and 40 % 4-methylphenol [33]. In this carcinogenesis study, groups of 50 rats were fed diets containing the mixture at 0, 1,500, 5,000 or 15,000 ppm (≈ 70, 230, or 720 mg/kg bw/d) for 105 weeks. Under the conditions of the study increased incidences of non-neoplastic lesions in the kidney (hyperplasia), nose (inflammation, hyperplasia and metaplasia) and liver (eosinophilic focus) were noted. The LOAEL was the lowest average daily dose, ≈ 70 mg/kg bw/d.

In summary, while protocols vary, results from repeated-dose testing employing 28-day to several 100-day exposures provide results similar to those observed in the 90-day oral gavage studies, with NOAEL values of between 100 and 60 mg/kg bw/d.

4.7 Toxicophores

As demonstrated in Table 5 of the supplemental information, based on *in silico* predictions, the alkylphenols triggered the repeated dose toxicity (HESS), protein binding for chromosomal

aberration and oestrogen receptor (ER) binding profilers within the OECD QSAR Toolbox v3.3.5 [34]. The alkylphenols analogues are associated with the presence of “phenols” or “substituted phenols” alerts, specifically with: phenols (mucous membrane irritation) Rank C, substituted phenols - Michael addition to the quinoid type and weak binder-OH. Additionally, the *para* substituted alkylphenols were assigned by p-alkylphenols (Hepatotoxicity) Rank A alert. ER - binding is also confirmed for 11 alkylphenols by the *in silico* nuclear receptor binding profiler [7]. This is not surprising as alkylphenols are associated with ER-mediated responses [35]. In summary, however, it is not clear how the toxicophores triggered are relevant to the endpoint discussed in this case study.

4.8 Mechanistic plausibility

Currently, there is no direct evidence for a common mechanism or mode of toxic action for mono-alkylphenols in mammals. However, in acute aquatic exposures to fish, alkylphenols are considered to act via the polar narcosis mode of action [36]. Bradbury et al. [37], expanding upon fish acute toxicity syndromes (FATS), derived a physiological/biochemical response set for defining toxicity of polar narcotics. Briefly, the characteristic whole fish responses to exposure to model polar narcotics, including phenol and 2,4-dimethylphenol, were tremors, initiated by a cough, that progressed to seizures and were followed by general depression and respiratory-cardiovascular collapse. The major changes in the respiratory-cardiovascular status upon exposure to polar narcotics were an increased cough frequency (in association with the seizures), and alterations in blood chemistry parameters attributed to the increased muscular activity causing a rapid shift toward anaerobic metabolism. These effects were found to be reversible and mimic the response to cataleptic anaesthetics [37], in which the animal passes through an excitatory phase

before progressing to general central nervous system depression. These finding form the basis for a presumptive Adverse Outcome Pathway.

The repeated-dose data summarized in Sections 4.6.1 and 4.6.2 are consistent. 90-day repeated oral exposure to short-chain mono-alkylphenols at high doses (>400 mg/kg bw/ d) lead to behavioural effects (e.g., tremors and then lethargy). LOAEL values (≈ 150 mg/kg bw/d) are typically based on a decrease in body weight. Subsequent histopathology findings, when noted, are minimal and considered likely secondary to the decreased weight gains. Increased relative kidney and liver weights, when noted, are likely compensatory to metabolism. NOAEL values are typically between 50 and 100 mg/kg bw/d. These findings, and a lack of organ-specific systemic toxicity, are consistent with what would be expected with phenols eliciting the polar narcosis mode of toxic action.

4.9 Relevant *in vitro* and *in silico* data

Within the US EPA toxicity forecaster program (ToxCast) [38], high through-put molecular screening data are available for a number of alkylphenols, specifically for 16 derivatives of this case study. These data are summarised in Table 3, a detailed list of the assays with active results is given in Supplementary material - Table 4.

Table 3. Summary of ToxCast Data for alkyl-substituted phenols

2-methylphenol	602 (2 active)
3-methylphenol	249 (3 active)
4-methylphenol	602 (4 active)
2-ethylphenol	250 (9 active)
3-ethylphenol	249 (8 active)
4-ethylphenol	250 (5 active)
4-propylphenol	250 (19 active)
2-isopropylphenol	250 (11 active)
3-isopropylphenol	250 (10 active)

4-isopropylphenol	247 (18 active)
4-butylphenol	250 (31 active)
2-secbutylphenol	602 (12 active)
4-secbutyphenol	603 (34 active)
2-tertbutylphenol	602 (22 active)
3-tertbutylphenol	250 (18 active)
4-tertbutylphenol	600 (31 active)

A trend is observed in the data reported in Table 3; generally, there is an increase in the number of ToxCast positive assays with the increase in size of the alkyl-chain.

From the assay-specific results reported in Table 4 in Supplementary material, it is clear that of the 66 different positive results only six assays are commonly activated by the majority of derivatives in the category. These six assays can be summated into two groups: Pregnane X receptor (PXR)-binding and ER-binding.

The PXR is a ligand-activated enhancer protein that is a member of the steroid/nuclear receptor super-family. Its primary function is to sense the presence of toxicant substances and in response to up-regulation of the expression of proteins involved in detoxification and clearance of these substances from the body [39]. PXR is activated by a large number of chemicals including hydroxylated ringed structures such as steroids. PXR activation induces the Phase I oxidative enzyme, CYP3A4 [40]. Falkner et al. noted it also upregulates the expression of Phase II conjugating enzymes (e.g., glutathione S-transferase) [41].

The oestrogen receptor (ER) is another ligand-activated enhancer protein that is a member of the steroid/nuclear receptor super-family. It mediates most of the biological effects of oestrogens at the level of gene regulation. It is an extremely well-studied receptor [42, 43]. In mammals, ER is encoded by two genes: alpha and beta (ERα and ERβ). Both genes function as signal transducers and transcription factors to modulate expression of other genes. Briefly, the oestrogen response elements (EREs) have highly varied affinity for hydroxylated ringed compounds (e.g., 17β-estradiol, nonylphenol). Estrodiol mimickers have been correlated with reproductive toxicity [44, 45].

Neither PXR-binding nor ER-binding is considered relevant to repeated-dose toxicity as the AOP effect for this read across is mortality, which is not the adverse effect associated with the PXR-binding or ER-binding. Therefore, the ToxCast data do not discredit the hypothesis that the

category members induce the polar narcosis mode of toxic action associated with mortality during a 90-day exposure regime.

The mono-alkylphenols in Table 1 were screened with a variety of *in silico* profilers [7, 34]; the positive results can be seen in Table 5 of Supplementary material. Briefly, the evaluation of potential binding to the receptors is based on structural fragments and physico-chemical features that have been identified as essential to bind to these nuclear receptors and induce a response. Specifically, profilers for nuclear receptor binding were run to identify potential binding to the following nuclear receptors: PPARs (peroxisome proliferator-activated receptors), AR (androgen receptor), AHR (aryl hydrocarbon receptor), ER (oestrogen receptor), GR (glucocorticoid receptor), PR (progesterone receptor), FXR (farnesoid X receptor), LXR (liver X receptor), PXR (pregnane X receptor), THR (thyroid hormone receptor), VDR (vitamin D receptor) as well as RAR/RXR (retinoic acid receptor/ retinoid X receptor). Outside of ER-binding, no potential receptor binding was predicted. Weak ER- binding was also identified for all analogues by profiler within OECD QSAR Toolbox v3.3.5. Note that ToxCast also tested for all of these receptors, and all assays other than those related to ER- and PXR were also negative.

Two additional profilers (protein binding for chromosomal aberration and repeated dose toxicity (HESS)) within OECD QSAR Toolbox were triggered by all or selected alkylphenols. The alerts are associated with the presence of phenol moiety and it is not clear how relevant they are to sub-chronic repeated-dose endpoint.

Taken collectively, the *in vitro* and *in silico* findings, which indicate no activity associated with specific receptors, are not inconsistent with the cited *in vivo* data, which indicates lethality during repeated oral-dose toxicity studies with short-chain mono-alkylphenols and are likely due to polar narcosis. Further supporting this conclusion responses observed in the repeated oral toxicity studies are consistent with responses associated with cataleptic anaesthetics.

5. Statement of uncertainty

The categorical assessments of uncertainties along with summary comments are presented in Tables 4 and 5. Short-chain, mono-alkylphenols are a category with acceptable data uncertainty and robust strengths-of-evidence for repeated-dose toxicity. Briefly, chemical similarity is high, and data uncertainty associated with the similarities in toxicokinetic, as well as toxicodynamic

profiles is low. Uncertainty associated with mechanistic relevance and completeness of the read-across is acceptable. These simple alkylphenols are thought to be associated with the polar narcosis mechanisms of toxicity. This molecular mechanism is well-studied, but not well-understood and no adverse outcome pathway is currently available. While it is unclear if oral repeated-dose toxicity is related to this mechanism, no evidence was found to suggest that it is not. Weight-of-evidence associated with the fundamentals of toxicokinetic, and toxicodynamic is high (i.e., this is a well-tested and well-understood group of chemicals). In terms of chemistry, the narrowly defined applicability domain of this category leads to all analogues or category members being highly similar. While there are differences among the category members with respect to physico-chemical properties, these differences are not considered toxicologically relevant.

Table 4. Data uncertainty and weight-of-evidence associated with the fundamentals of chemical, transformation/toxicokinetic and toxicological similarity**.**

Similarity Parameter	Data Uncertainty [a] (empirical, modelled) (low, medium, high)	Strength of Evidence [b] (low, medium, high)	Comment
Substance Identification, Structure and Chemical Classifications	Low	High	All category members have CAS numbers. All members are structurally highly similar. Specifically, they: 1) belong to a common chemical class, phenols and the subclasses alkyl phenols and mono-substituted phenols 2) possess a common molecular scaffolding, a benzene backbone. Structurally, the main variables are the shape and size of the alkyl-substituents and its position on the phenolic ring.
Physico-Chemical & Molecular Properties	Empirical: Low Modelled: Low	High	All category members are appropriately similar with respect to key physico-chemical and molecular properties. A large portion of their physico-chemical properties have been determined experimentally and calculated values can be taken with high confidence. Properties values, with the exception of density, tread in relation to C-atom number within a scaffold. Specifically, all category members exhibit molecular weights from ≈100 to150 g/mol. While hydrophobicity (log Kow) increases with number of C-atoms from ≈2.00 to ≈3.50, density and pKa are constant at ≈1.0 g/cm^3 and ≈10, respectively. Vapour pressure and water solubility, while influenced by position of substituted (e.g., *para* > than *ortho*) decrease with substituent size. With the exception of 4-secbutyl and 4-tertbutyl, melting point varies for 12 to 50 °C**.** Boiling point varies for 190 to 250 °C.
Substituents, Functional Groups, & Extended Structural Fragments	Low	High	Substituents and functional groups are consistent across all category members. Specifically, all members have common constituents in the form of: 1) a benzene ring, 2) single functional polar group, -OH, and 3) and alkyl structural fragments, -H, -CH_3 -CH_2-CH_3, etc. There are no extended structural fragments.

Similarity Parameter	Data Uncertainty [a] (empirical, modelled) (low, medium, high)	Strength of Evidence [b] (low, medium, high)	Comment
Toxicokinetics Similarity	Low: (3 source studies)	High: 2 additional studies	Based on *in vivo* studies for multiple category members, there is evidence for similar toxicokinetics and metabolic pathways. Specifically, small alkyl phenols are readily absorbed by the oral routes. The portal-of-entry metabolism is extensive and involves sulphate and glucuronide conjugations. Once absorbed, alkyl phenols are distributed in the body, with levels (on a per-gram-tissue basis) in liver and kidney reported as being higher than in other tissues. Elimination from the body is rapid, primarily as sulphate and glucuronide conjugates in the urine. Alkyl phenols do not appear to accumulate significantly in the body.
In vivo Toxicodynamic Similarity	Empirical: *In vivo*: Low (5 source substances)	High: 9 additional studies	Based on *in vivo* studies for multiple category members, there is evidence for similar repeated-dose toxicodynamics. Specifically, at higher doses 90-day repeated oral gavage exposure result displays of lethargy, tremors, etc. Dose-dependent body weight decrease is observed at intermediate doses. Histopathology findings, when noted, were minimal and considered likely secondary to the decreased weight gains. Increased relative kidney and liver weights, when noted, are likely compensatory to metabolism.
Toxicophores /Mechanistic alerts	Low	Medium	Based on *in silico* profilers, no category member contains any established toxicophores other than polar narcosis or ER-binding.
Mechanistic plausibility and AOP-Related Events	Low-to-Medium-	Low-to-Medium	No AOP is currently available for the hypothesized mode of action, polar narcosis; *in vivo* data is not inconsistent with the proposed mode of action.
Relevant *in vitro* and *in silico* endpoints	Low	Medium	*In vitro* data in the form of ToxCast and *in silico* data in the form of screening profilers finds little outside of ER-binding affinity to be common among the category members.
Overall uncertainty in similarity of category members is low. Summary: Key features of chemistry are similar within the category. Key features of toxicokinetics and metabolism are generally common within the category. Key features of toxicodynamics are generally common within the category. Positive features of mechanistically similarity? are generally lacking.			

[a] Uncertainty associated with underlying information/data used in the exercise
[b] Consistency within the information/data used to support the similarity rational and prediction

From a toxicokinetic standpoint, data for 2-methyl- and 3-methyl-phenol, as well as 4-tertbutylphenol is supplemented with data for a mixture of 3- and 4-methylphenol, as well as phenol and 2-isopropl-5-methylphenol. All substances are readily absorbed orally, metabolised via phase II conjugations and eliminated rapidly in the urine.

From a toxicodynamic standpoint, the experimental 90-day oral gavage data for 2-methyl-, 3-methyl- and 4-methylphenol is supplemented by other experimental repeated dose data. Included in this are data for 4-tertbutylphenol, 2-secbutylphenol 2-tertbutylphenol, a mixture of 3- and 4-

methylphenol, mixture of 2-, 3-, and 4-ethylphenols, as well as a mixture of various isomers of xylenols. Collectively these data suggest that all category members are toxicodynamically similar, both qualitatively (symptomology) and quantitatively (potency).

The major source of uncertainty for this group of alkylphenols is associated with mechanistic plausibility. There is no adverse outcome pathway related to repeated-dose toxicity associated with this category.

Table 5. Assessment of uncertainty associated with mechanistic relevance and completeness of the read-across.

Factor	Uncertainty or WoE (low, medium, high)	Comment
The problem and premise of the read-across	Medium	The endpoint to be read across, oral gavage sub-chronic repeated-dose toxicity, for mono-alkylphenols is moderately well-studied but not well-understood.
***In vivo* data read across**		
Number of analogues in the source set	Low; 6 of 24 tested	There are several suitable members in each of the two sub-categories with *in vivo* apical endpoint data usable for read-across.
Quality of the *in vivo* apical endpoint data read across	Low; consistent LOAEL symptoms; similar NOAEL potency; several supporting studies	High quality empirical data from TG 408 for the stated regulatory endpoint are available. Additional *in vivo* data (i.e., TG 407 and TG 422) exist for other alkyl-substituted phenols.
Severity of the apical *in vivo* hazard	Low-to-Medium; The most common reported NOAEL value is 50 mg/kg bw/d.	Typically, gavage exposure scheme leads to high potency that exposure via fed or drinking.
Evidence to the biological argument for RA		
Robustness of analogue data set	Low; The *in vivo* repeated-dose toxicity data is adequate. The *in vitro* and *in silico* data for alkyl-substituted phenols is consistent.	The *in vivo* studies were judged to be reliable and conducted under the appropriate conditions. Relevant *in vitro* data is limited.
Concordance with regard to the intermediate and apical effects and potency data	Medium to High; intermediate effects data are very limited.	Since there is no toxicity pathway for alkylphenols repeated-dose effects, there are no true intermediate events. Without relevant *in vitro* data, concordance between events cannot be ascertained.
Weight-of-Evidence (WoE)	High	Overall the available information and data is generally consistent with the stated premise. The structural limitations of the category strengthen the WoE. Having multiple sources of toxicokinetics data strengthens the WoE. Having multiple sources of *in vivo* data adds to the WoE. The only consistent results from ToxCast, and receptor binding screening (i.e., ER- and PXR-related activity, does not appear to be related to repeated-dose toxicity and therefore has no impact on WoE.

The overall uncertainty associated with this read-across prediction is judged to be medium largely due to mechanistic uncertainty.

6. Conclusions

In vivo oral repeated-dose exposure to alkyl-substituted phenols gives rise to a variety of toxicity symptoms which are dose dependent. At high doses (>400 mg/kg bw/ d) behavioural effects (e.g., lethargy, tremors, etc.) are observed LOAEL values in the range of 75 to 175 mg/kg bw/d are typically based on a decrease in body weight. Adverse effects associated with liver and/or kidney are not physiologically significant and considered secondary to the decreased weight gains and likely compensatory in nature. A NOAEL value of 50 mg/kg bw/d may be read across to fill data gaps for the other derivatives in the category.

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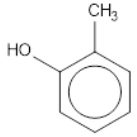
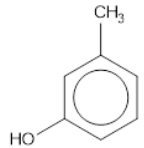
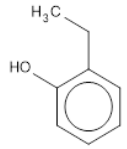
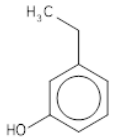
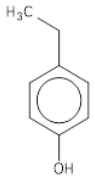
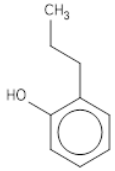
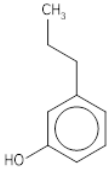
Supplementary Material

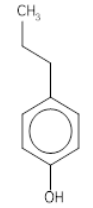
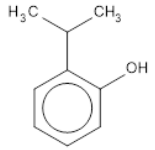
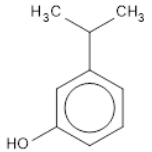
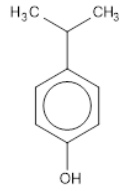
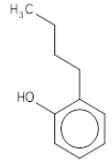
Read-Across of 90-Day Rat Oral Repeated-Dose Toxicity: A Case Study for Selected mono-alkylphenols

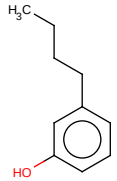
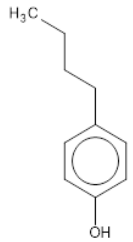
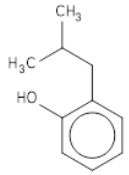
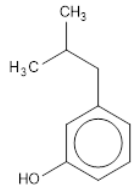
Tables for Assessing Similarity of Analogues and Category Members for Read-Across

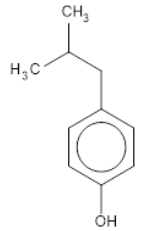
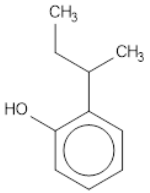
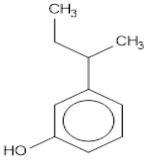
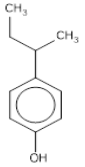
Table 1: Comparison of Substance Identification, Structure and Chemical Classifications

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula	Molecular Weight [g/mol]
1	2-methylphenol	95-48-7	Cc1ccccc1O		C7H8O	108
2	3-methylphenol	108-39-4	Cc1cccc(O)c1		C7H8O	108
3	4-methylphenol	106-44-5	Cc1ccc(O)cc1		C7H8O	108

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula	Molecular Weight [g/mol]
4	2-ethylphenol	90-00-6	CCc1ccccc1O		C8H10O	122
5	3-ethylphenol	620-17-7	CCc1cccc(O)c1		C8H10O	122
6	4-ethylphenol	123-07-9	CCc1ccc(O)cc1		C8H10O	122
7	2-propylphenol	644-35-9	CCCc1ccccc1O		C9H12O	136
8	3-propylphenol	621-27-2	CCCc1cccc(O)c1		C9H12O	136

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula	Molecular Weight [g/mol]
9	4-propylphenol	645-56-7	CCCc1ccc(O)cc1		C9H12O	136
10	2-isopropylphenol	88-69-7	c1(c(cccc1)O)C(C)C		C9H12O	136
11	3-isopropylphenol	618-45-1	CC(C)c1cccc(O)c1		C9H12O	136
12	4-isopropylphenol	99-89-8	CC(C)c1ccc(O)cc1		C9H12O	136
13	2-butylphenol	3180-09-4	CCCCc1ccccc1O		C10H14O	150

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula	Molecular Weight [g/mol]
14	3-butylphenol	28805-86-9	CCCCc1cccc(c1)O		C10H14O	150
15	4-butylphenol	1638-22-8	CCCCc1ccc(O)cc1		C10H14O	150
16	2-isobutylphenol	4167-75-3	CC(C)Cc1ccccc1O		C10H14O	150
17	3-isobutylphenol	30749-25-8	CC(C)Cc1cccc(O)c1		C10H14O	150

ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula	Molecular Weight [g/mol]
18	4-isobutylphenol	4167-74-2	CC(C)Cc1ccc(O)cc1		C10H14O	150
19	2-secbutylphenol	89-72-5	CCC(C)c1ccccc1O		C10H14O	150
20	3-secbutylphenol	3522-86-9	CCC(C)c1cccc(O)c1		C10H14O	150
21	4-secbutyphenol	99-71-8	CCC(C)c1ccc(O)cc1		C10H14O	150

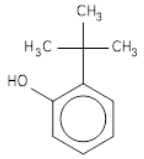
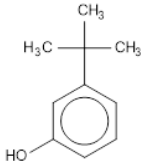
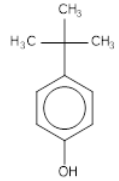
ID	Name	CAS No:	SMILES	2D Structure	Molecular Formula	Molecular Weight [g/mol]
22	2-tertbutylphenol	88-18-6	CC(C)(C)c1ccccc1O		C10H14O	150
23	3-tertbutylphenol	585-34-2	CC(C)(C)c1cccc(c1)O		C10H14O	150
24	4-tertbutylphenol	98-54-4	CC(C)(C)c1ccc(cc1)O		C10H14O	150

Table 2: Comparison of Physico-Chemical and Molecular Properties[1]

ID	Name	Molecular Weight [g/mol]	Log Kow	Vapor Pressure [Pa at 25°C]	Density[c] [g/cm^3]	Melting Point [°C]	Water Solubility [mg/L]	Boiling Point [°C]	pKa
1	2-methylphenol	108	1.95[a]	33.40	1.14	31	9066	191	10.3
2	3-methylphenol	108	1.96[a]	22.3	1.04	12	8890	201	10.1
3	4-methylphenol	108	1.94[a]	16.60	1	15.96	9246	191	10.3
4	2-ethylphenol	122	2.47[a]	19.7	1	18	2912	205	10.2[d]
5	3-ethylphenol	122	2.4[a]	9.15	1	27.13	3342	211	9.9[d]
6	4-ethylphenol	122	2.58[a]	5.17	1	27.13	4900	211	10[e]
7	2-propylphenol	136	2.93[a]	8.37	1	38.3	1039	230	10.5[f]
8	3-propylphenol	136	3.04	4.9	1	26	1669	228	10.1[f]
9	4-propylphenol	136	3.2[b]	4.13	1	22	1280	233	10.3[f]
10	2-isopropylphenol	136	2.88[a]	12	1	15.5	1146	214	10.5[f]
11	3-isopropylphenol	136	2.97	4.9	1	26	962.3	228	10.2[f]
12	4-isopropylphenol	136	2.9[a]	2.01	1	27.49	1102	230	10.2[f]
13	2-butylphenol	150	3.53	0.027	1	49.21	276.4	235	10.6
14	3-butylphenol	150	3.27[a]	5.35	1	16	464.0	228	10.6
15	4-butylphenol	150	3.65[a]	1.17	1	22	219.8	248	10.1
16	2-isobutylphenol	150	3.46	2.31	1	38.56	319.4	237	10.2
17	3-isobutylphenol	150	3.46	2.31	1	38.56	319.4	237	10.0
18	4-isobutylphenol	150	3.46	2.31	1	38.56	319.4	237	9.8
19	2-secbutylphenol	150	3.27[a]	5.35	0.98	38.56	464	237	10.4
20	3-secbutylphenol	150	3.46	2.13	1	38.56	319.4	237	10.0
21	4-secbutyphenol	150	3.08[a]	1.12	1	22	674.2	241	10.1
22	2-tertbutylphenol	150	3.31[a]	0.98	1	36.91	428.9	223	10.3[g]
23	3-tertbutylphenol	150	3.3[b]	1.8	1	42.3	437.4	240	10.1
24	4-tertbutylphenol	150	3.3	0.00447	1	36.91	580.0	237	10.4

[1]Values typically derived from EPISuite v4.1 experimental values where taken over predicted when available; [a] Hansch, C et al. (1995), [b] Sangster (1993), [c] ACD/Lab Percepta Platform - PhysChem Module (from ChemSpider), [d] Pearce,P.J. & Simkins,R.J.J. (1968), [e] Schultz,T.W. (1987A), [f] Serjeant,E.P. & Dempsey,B. (1979), [g] Schueuerman,G. (1991).

Table 3: Comparison of Substituents, Functional Groups, and Extended Structural Fragments.

ID	Name	Key Substituent(s)	Functional Group(s)				Chemical Class:
1	2-methylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH3]	Aromatic Carbon [C]		alkyl phenols
2	3-methylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH3]	Aromatic Carbon [C]		alkyl phenols
3	4-methylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH3]	Aromatic Carbon [C]		alkyl phenols
4	2-ethylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]		alkyl phenols
5	3-ethylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]		alkyl phenols
6	4-ethylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH2] [CH3]	Aromatic Carbon [C]		alkyl phenols
7	2-propylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH] [CH2] [CH3]	Aromatic Carbon [C]		alkyl phenols
8	3-propylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH][CH2] [CH3]	Aromatic Carbon [C]		alkyl phenols
9	4-propylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH][CH2] [CH3]	Aromatic Carbon [C]		alkyl phenols
10	2-isopropylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH] [CH3]	Aromatic Carbon [C]	Tertiary Carbon	alkyl phenols
11	3-isopropylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH] [CH3]	Aromatic Carbon [C]	Tertiary Carbon	alkyl phenols
12	4-isopropylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH] [-CH3]	Aromatic Carbon [C]	Tertiary Carbon	alkyl phenols
13	2-butylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH][CH2] [CH3]	Aromatic Carbon [C]		alkyl phenols
14	3-butylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH][CH2] [CH3]	Aromatic Carbon [C]		alkyl phenols
15	4-butylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH][CH2] [CH3]	Aromatic Carbon [C]		alkyl phenols
16	2-isobutylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH][CH2] [CH3]	Aromatic Carbon [C]	Tertiary Carbon	alkyl phenols
17	3-isobutylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH][CH2] -CH3]	Aromatic Carbon [C]	Tertiary Carbon	alkyl phenols
18	4-isobutylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH][-CH2-] [-CH3]	Aromatic Carbon [C]	Tertiary Carbon	alkyl phenols
19	2-secbutylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH][CH2] [CH3]	Aromatic Carbon [C]	Tertiary Carbon	alkyl phenols

20	3-secbutylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH][CH2] [CH3]	Aromatic Carbon [C]	Tertiary Carbon	alkyl phenols
21	4-secbutyphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH][CH2] [CH3]	Aromatic Carbon [C]	Tertiary Carbon	alkyl phenols
22	2-tertbutylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH][C] [CH3]	Aromatic Carbon [C]	Tertiary Carbon	alkyl phenols
23	3-tertbutylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH][C] [CH3]	Aromatic Carbon [C]	Tertiary Carbon	alkyl phenols
24	4-tertbutylphenol	phenol ($C_6H_{12}O$)	Alcohol, olefinic attach [-OH]	Aliphatic Carbon [CH][C] [CH3]	Aromatic Carbon [C]	Tertiary Carbon	alkyl phenols

Table 4: Toxcast assay active results for derivatives.

Name / Toxcast assay	2-methylphenol	3-methylphenol	4-methylphenol	2-ethylphenol	3-ethylphenol	4-ethylphenol	4-propylphenol	2-isopropylphenol	3-isopropylphenol	4-isopropylphenol	4-butylphenol	2-secbutylphenol	4-secbutylphenol	2-tertbutylphenol	3-tertbutylphenol	4-tertbutylphenol
ACEA_T47D_80hr_Negative	*	*	*	*	*	*	*	*	*	*	68.8	*	54.7	*	*	*
ACEA_T47D_80hr_Positive	*	71.7	*	*	*	*	64.8	*	*	20.7	7.19	*	9.68	*	*	*
ATG_Ahr_CIS	*	*	*	17.8	*	*	*	*	*	*	*	*	*	*	*	*
ATG_Ahr_CIS_perc	*	*	*	17.8	*	*	*	*	*	*	*	*	*	*	*	*
ATG_AP_1_CIS	112	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
ATG_AP_1_CIS_perc	112	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
ATG_BRE_CIS	*	*	*	*	*	*	*	*	*	*	*	*	96.5	*	*	*
ATG_BRE_CIS_perc	*	*	*	*	*	*	*	*	*	*	*	*	96.5	*	*	*
ATG_CMV_CIS	*	*	*	*	*	*	*	*	*	*	79.6	*	86.5	*	*	*
ATG_CMV_CIS_perc	*	*	*	*	*	*	*	*	*	*	79.6	*	86.5	*	*	*
ATG_CRE_CIS	*	*	*	*	*	*	*	*	*	*	*	*	*	52.4	*	*
ATG_CRE_CIS_perc	*	*	*	*	*	*	*	*	*	*	*	*	*	52.4	*	*
ATG_ERa_TRANS	*	*	40	*	153	86	14.5	114	89	28.2	6.81	68.3	8.19	24.5	24.4	7.66
ATG_ERa_TRANS_perc	*	*	8.14	*	144	62.7	11.2	111	61	22.3	4.91	28.6	3.82	17.8	12.3	2.37
ATG_ERE_CIS	*	*	*	*	*	*	*	*	*	*	11.5	*	4.76	*	*	5.78
ATG_ERE_CIS_perc	*	*	*	*	*	*	*	*	*	*	7.73	*	3.27	*	*	3.65
ATG_HSE_CIS	*	*	*	*	*	*	*	*	*	*	116	*	*	*	*	*
ATG_HSE_CIS_perc	*	*	*	*	*	*	*	*	*	*	116	*	*	*	*	*
ATG_MRE_CIS	*	*	*	*	*	*	*	*	*	*	*	*	*	120	*	149

ATG_MRE_CIS_perc	*	*	*	*	*	*	*	*	*	*	*	*	*	120	*	149
ATG_NRF2_ARE_CIS	*	*	*	39.2	*	*	32.5	*	*	62	90.9	73.4	87.8	*	*	104
ATG_NRF2_ARE_CIS_perc	*	*	*	39.2	*	*	32.5	*	*	62	90.9	73.4	87.8	*	*	104
ATG_NURR1_TRANS	*	*	*	*	*	*	*	*	*	*	*	*	*	60.2	*	*
ATG_NURR1_TRANS_perc	*	*	*	*	*	*	*	*	*	*	*	*	*	60.2	*	*
ATG_PPARa_TRANS	*	*	*	*	*	*	*	*	*	*	*	*	11.6	*	*	*
ATG_PPARa_TRANS_perc	*	*	*	*	*	*	*	*	*	*	*	*	11.6	*	*	*
ATG_PPARg_TRANS	*	*	*	*	*	*	*	*	*	*	78.1	*	70.9	*	*	*
ATG_PPARg_TRANS_perc	*	*	*	*	*	*	*	*	*	*	78.1	*	70.9	*	*	*
ATG_PXR_TRANS	*	*	*	18.7	*	*	26.1	*	53	29.4	83.1	35.1	26.8	26.1	29.2	17.4
ATG_PXR_TRANS_perc	*	*	*	18.7	*	*	26.1	*	53	29.4	83.1	35.1	26.8	26.1	29.2	17.4
ATG_PXRE_CIS	*	*	*	25.6	44.9	*	37.4	99.9	22.2	*	*	35.1	33.1	27.7	29.4	27.3
ATG_PXRE_CIS_perc	*	*	*	25.6	44.9	*	37.4	99.9	22.2	*	*	35.1	33.1	27.7	29.4	27.3
ATG_RORb_TRANS	*	*	*	*	*	*	*	*	*	*	*	*	16.2	*	*	*
ATG_RORb_TRANS_perc	*	*	*	*	*	*	*	*	*	*	*	*	16.2	*	*	*
ATG_RXRb_TRANS	*	*	*	*	*	*	41.4	94.6	*	84.3	87.7	*	49.9	26.6	96.4	*
ATG_RXRb_TRANS_perc	*	*	*	*	*	*	41.4	94.6	*	84.3	87.7	*	49.9	26.6	96.4	*
ATG_VDRE_CIS	*	*	*	*	*	*	*	*	*	*	*	101	*	*	*	23.6
ATG_VDRE_CIS_perc	*	*	*	*	*	*	*	*	*	*	*	101	*	*	*	23.6
NVS_GPCR_hV1A	*	NA	22.5	NA	NA	NA	NA	NA	NA	NA	NA	*	*	*	NA	*
NVS_MP_rPBR	*	*	*	*	36.1	*	*	*	*	*	*	*	*	*	*	*
NVS_NR_rMR	*	*	*	*	*	10.6	*	*	*	*	*	*	*	*	*	*
NVS_TR_hNET	*	NA	*	NA	NA	NA	NA	NA	NA	NA	NA	*	10.3	*	NA	*
OT_ER_ERaERa_0480	*	*	*	*	*	*	47.1	*	*	35.2	15.2	*	37.3	*	44.2	40.4
OT_ER_ERaERa_1440	*	*	*	*	*	*	*	*	*	*	17.3	*	48.2	*	*	40
OT_ER_ERaERb_0480	*	*	*	*	*	44.5	37.2	*	38.4	40.5	11.5	*	32	*	36.7	26.4
OT_ER_ERaERb_1440	*	*	*	*	*	*	41	*	*	46.4	14	*	33.9	*	45.7	24
OT_ER_ERbERb_0480	*	*	*	*	*	*	35.4	*	44	43.7	11.2	*	15.3	41.9	38.1	23.8
OT_ER_ERbERb_1440	*	*	*	*	*	*	39.5	*	35.3	41.1	14	*	28.4	*	41.5	18.6
OT_ERa_EREGFP_0120	*	*	*	*	*	*	*	*	*	*	*	*	10.3	*	*	10.6

OT_ERa_EREGFP_0480	*	*	*	*	*	*	*	*	*	*	*	*	18.7	*	*	11.1
OT_NURR1_NURR1RXRa_0480	*	*	*	*	*	*	*	*	*	*	68.2	*	*	*	*	*
OT_SRC1_SRC1FXR_0480	*	*	*	*	*	*	*	*	*	*	*	*	*	*	48.5	*
OT_SRC1_SRC1FXR_1440	*	*	63.7	*	*	*	*	*	*	*	*	*	*	*	34.2	*
Tox21_AhR_viability	*	*	*	*	*	*	*	50.9	*	0.001	*	*	*	*	*	*
Tox21_AR_BLA_Agonist_ch1	*	0.017	*	*	*	*	*	*	*	*	*	*	*	*	*	0.002
Tox21_AR_LUC_MDAKB2_Antagonist_viability	*	*	*	*	*	*	*	22.9	*	*	*	*	*	66.3	*	59.6
Tox21_Aromatase_Inhibition	*	*	*	*	*	*	*	*	*	*	38.9	*	*	*	*	*
Tox21_Aromatase_Inhibition_viability	*	*	*	*	*	*	*	53.4	*	0.641	*	*	*	10.6	*	39.5
Tox21_ELG1_LUC_Agonist_viability	*	*	*	*	22.7	*	*	*	*	*	*	*	*	*	*	*
Tox21_ERa_BLA_Agonist_ch2	*	*	*	*	*	*	*	*	*	*	*	*	*	48.9	*	47.6
Tox21_ERa_BLA_Agonist_ratio	*	*	*	*	0.13	*	*	*	*	*	*	*	*	51.6	*	45.4
Tox21_ERa_LUC_BG1_Antagonist_viability	*	*	*	*	*	*	*	*	*	0.001	*	*	*	*	*	*
Tox21_MitochondrialToxicity_ratio	*	*	*	*	*	*	42.6	*	*	*	44.2	37.3	28.4	50.2	40.5	25.4
Tox21_MitochondrialToxicity_rhodamine	*	*	*	*	*	*	43.8	*	*	*	45.7	49.3	29.6	50.4	41.9	24.5
Tox21_PPARg_BLA_Agonist_ch1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1E-03
Tox21_TR_LUC_GH3_Antagonist_viability	*	*	*	*	*	*	*	9.59	*	*	58.7	*	*	12.5	*	12.9

Table 5: Comparison of Toxicophores**.**

ID	Name	Protein binding for chromosomal aberration by OECD[1]	Repeated dose toxicity (HESS) by OECD[1]	Oestrogen Receptor binding by OECD[1]	Nuclear receptor binding[2]
1	2-methylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	No alert
2	3-methylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	No alert
3	4-methylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C p-Alkylphenols (Hepatotoxicity) Rank A	Weak binder, OH	No alert
4	2-ethylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	No alert
5	3-ethylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	No alert
6	4-ethylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C p-Alkylphenols (Hepatotoxicity) Rank A	Weak binder, OH	No alert
7	2-propylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	No alert
8	3-propylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	No alert
9	4-propylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C p-Alkylphenols (Hepatotoxicity) Rank A	Weak binder, OH	No alert

ID	Name	Protein binding for chromosomal aberration by OECD[1]	Repeated dose toxicity (HESS) by OECD[1]	Oestrogen Receptor binding by OECD[1]	Nuclear receptor binding[2]
10	2-isopropylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	No alert
11	3-isopropylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	No alert
12	4-isopropylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C p-Alkylphenols (Hepatotoxicity) Rank A	Weak binder, OH	No alert
13	2-butylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	No alert
14	3-butylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	ER
15	4-butylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C p-Alkylphenols (Hepatotoxicity) Rank A	Weak binder, OH	ER
16	2-isobutylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	ER
17	3-isobutylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	ER
18	4-isobutylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C p-Alkylphenols (Hepatotoxicity) Rank A	Weak binder, OH	ER
19	2-secbutylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	ER

ID	Name	Protein binding for chromosomal aberration by OECD[1]	Repeated dose toxicity (HESS) by OECD[1]	Oestrogen Receptor binding by OECD[1]	Nuclear receptor binding[2]
20	3-secbutylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	ER
21	4-secbutyphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C p-Alkylphenols (Hepatotoxicity) Rank A	Weak binder, OH	ER
22	2-tertbutylphenol	No alert	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	ER
23	3-tertbutylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C	Weak binder, OH	ER
24	4-tertbutylphenol	Substituted Phenols	Phenols (Mucous membrane irritation) Rank C p-Alkylphenols (Hepatotoxicity) Rank A	Weak binder, OH	ER

[1] OECD QSAR Toolbox 3.3. [2] COSMOS profilers available via COSMOS space: http://cosmosspace.cosmostox.eu